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THERMAL SHOCKING OF BACTERIA FOR HIGH FLAVOR DEVELOPMENT IN THE MANUFACTURE OF CHEDDAR CHEESE

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Despite the fact that years of research have been dedicated to the factors influencing the ripening of Cheddar cheese, little is known which can contribute to the technical manufacture of palatable, highly flavored cheeses in a shorter period of time. In December, 1978, the Intersociety Research Committee of the Dairy Science, Animal Science, Poultry Science and Meat Science Associations, in cooperation with industry, government, and academia, identified 11 areas that must be emphasized to insure future availability of high-quality, economical foods from animals. The Committee identified Dairy Products Research as one of the key areas in need of immediate attention and one from which potential benefits could be derived. Within this research area, the main emphasis was placed on reducing the amount of energy used in the processing of dairy products. One of the research areas enumerated was the development of methods designed to reduce the time required to produce flavorful, ripened cheeses. This included the assessment of alternative methods for the manufacture of high quality cheese at lower cost, including measurement of microbial, enzymatic, and chemical changes during curing. This paper is a preliminary report of work directed toward the objectives described above.

The model system chosen was Cheddar cheese in which the fat content was reduced to 20-22%. While the availability of such reduced fat cheeses is desirable from the viewpoint of nutrition, lack of adequate flavor development and crumbly inferior body characteristics are commonly associated with the curing of such cheeses. As an approach to the problem of curing of nutritionally acceptable reduced-fat Cheddar cheese, the concept of thermal shocking of lactic cultures was applied. This concept was developed by Pettersson and Sjöstrom (1) in 1975 and was based on the differential heat stability of various key enzyme systems in lactic acid bacteria. Thus, exposure of lactic starter cultures to temperatures between 60 and 64°C for 15-16 seconds destroyed the activity of enzymes involved in lactic acid synthesis, while the enzymes responsible for fat and protein hydrolysis remained, for the most part, unaltered. Consequently, a palatable cheese could theoretically be manufactured.

As the first step in this study, the effect of heat on the survival and acid production of <u>Streptococcus cremoris</u> ATCC 14365 was evaluated (2). The culture was grown overnight, centrifuged, and resuspended in 10% nonfat dry milk at a concentration of 107 colony-forming-units per milliliter. The cell suspension was passed through a small heating coil that allowed for a 15-16-second residence time for the sample at the temperatures indicated in Table 1. These conditions approximated the high-temperature-short-time (HTST) pasteurization process. After the heat-shocking, the growth characteristics and acid production of the culture were followed for a period of 5 hours or longer.

Near 56°C, the point was approached where the culture was seriously damaged in its acid producing capability and in its ability to increase the live cell count (Table 1). At 56°C, the pH curve was flat, indicating permanent impairment of the enzyme systems involved in lactic acid synthesis. Also, after exposure to this temperature, even the surviving population of cells appeared to be dying off, as indicated by the diminishing number of colony-forming units over a period of 5 hours. Even at the relatively low temperature of 52°C, a small percentage of the population of \underline{S} . \underline{C} cremoris was destroyed; as the temperature increased, the percent loss in live \underline{C} count increased.

We also examined the heat stability of certain enzyme systems of \underline{S} . $\underline{cremoris}$. Heat shocking at 60° and 64°C resulted in a 15 and 30% loss of protease activity, respectively, as measured by casein hydrolysis. Examination of three peptide hydrolase enzymes showed that after a 15-16-second exposure to 64°C, 87% of alanylglycine hydrolase, 72% of leucylglycine hydrolase, and 85% of leucylalanine hydrolase activity survived. These findings confirmed that several enzymes believed to be involved in the development of desirable cheese flavor and physical characteristics retain a large percentage of activity following the heat treatment of \underline{S} . $\underline{cremoris}$ cell suspensions.

For cheese making, the S. cremoris culture was grown in a 14-liter fermentor. During growth, the culture was continuously neutralized with ammonium hydroxide. At the end of 16 hours, the cell count was 10^9 cfu/ml. The cells were collected by continuous flow centrifugation and the cell paste was dispersed in 200 gallons of milk with the fat content adjusted to 2 percent. The cell paste added to the milk yielded a final live count of 10^7 cfu/ml. The milk was then heat shocked at 64° C (147°F) for 16 seconds in a 10,000#/hour pilot plant HTST unit. After cooling to setting temperature, the milk was pumped into the vat, and a mixed strain direct-to-the vat starter culture was added at a level of 10^7 cfu/ml. Thus, the total number of bacterial cells, including live and heat-shocked cells, was in the range of 2 x 10^7 cfu/ml. The cheesemaking process followed a conventional Cheddar cheese manufacturing schedule. The 40# blocks were cured at 5° C (42° F). Cheese samples were taken and analyzed at monthly intervals.

Table 2 shows the compositional changes of the control and experimental cheeses as a function of time in storage. The cheeses were nearly identical in gross composition, that is, in fat, moisture and salt content. The pH of the experimental cheese, containing the thermally shocked S. cremoris cells, tended to register below that of the control during curing, although the total bacterial count per gram of cheese was not greater than that found in the untreated control. The amount of 5% trichloro-acetic acid-soluble material, as measured by absorbance at 280 nm, was always greater in the experimental cheese for the time in cure reported in Table 2. This indicates a greater degree of protein hydrolysis. Measurable dipeptidase activities were surprisingly low, and little difference was found between the control and experimental cheeses.

It was interesting, and perhaps of ultimate importance, to find significant differences in the amount of free fatty acids developing in the cheese. In the control cheese, after three months of curing, the total amount of free fatty acids increased by only 2.5 percent, whereas in the experimental cheese, they increased 58 percent. This dramatic increase in free fatty acid content was indicative of increased lipolytic activity. Table 3 shows the change in free fatty acid content of both control and experimental cheeses up to 4 months in storage. The rise in C_2 , C_4 and C_6 fatty acids in the experimental cheese was most significant because of their purported

le in cheese flavor development as precursors to other flavor compounds. Recent studies with full-fat cheese containing thermally shocked \underline{S} . $\underline{cremoris}$ at the same levels described herein verify the above findings. The occurrence of C_2 (acetic acid) is even more pronounced in experimental full-fat cheeses. Organoleptic evaluation of the experimental cheese showed it to have a more mature flavor than the untreated control; the increased concentration of free fatty acids did not result in the detection of lipolytic flavor.

This study has demonstrated that thermal shocking of lactic acid cultures leaves the bulk of proteolytic and lipolytic activities intact and that such cultures may be used for developing mature Cheddar flavor in reduced-fat cheese. In our opinion, this approach to the manufacture of reduced- and full-fat Cheddar and other types of cheeses merits, further consideration, especially since the reduction of fat intake has received such wide attention and because energy could be saved in the development of good Cheddar flavor in a shorter time. However, caution should be exercised in the selection of suitable strains of lactic culture which, when thermally shocked, produce the desired effects on both body and flavor of the manufactured product.

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Table 1

EFFECT OF HEAT ON STREPTOCOCCUS CREMORIS ATCC 14365

16 SEC HOLDING AT °C	% LOSS IN CFU/ML	pH AT 0 HR 5 HR		
52	18.3	6.49	6.27	
54	43.5	6.40	6.00	
56	54.2	6.49	6.42	
58	93 .9	6.39	6.38	
60	99.9	6.49	6.49	

Table 2
PROPERTIES OF REDUCED FAT CHEDDAR CHEESES

	O TIME		1 MONTH		2 MONTHS		3 MONTHS		4 MONTHS	
	c ^a	Ea	C	E	C	E	С	E	C	E
FAT	21.5%	21.0%							21.5	21.0
MOISTURE	44.4	44.4							44.4	44.4
SALT	1.3	1.15							1.3	1.15
pН	5.28	5.20	5.25	5,25	5.23	5.16	5.11	5.07	5.12	5.08
CFU/g x 10 ⁷	61	58	30	12.5	1.7	3.6	6.5	1.62	4.2	1.3
5% TCA SOLUBLE, A280b	0.60	0.60	1.59	2.04	2.04	2.24	2.44	2.72	2.64	2.85
μg FFA/g ^C	932	938	938	973	946	1,024	956	1,488	968	1,521

a C = control; E = experimental cheese.

b TCA = trichloroacetic acid.

c FFA = free fatty acid.

Table 3

FREE FATTY ACIDS (µg/g) IN REDUCED FAT CHEESE

ACID	, O TIME		1 MONTH		2 MONTHS		3 MONTHS		4 MONTHS	
	C ^a	Ea	C	Е	С	E	c	E	С	Е
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2.0									_	72
4.0	62	64	60	66	62	70	62	155	66	161
6.0	23	23	25	28	25	34	26	105	28	108
8.0	24	22	24	24	24	28	25	64	26	65
10.0	30	31	31	33	32	37	35	. 40	37	41
12.0	41	41	43	43	43	47	44	60	41	61
14.0	116	118	117	121	118	125	116	152	117	154
16.0	292	301	290	305	292	320	294	382	296	394
18.0	106	104	108	110	110	115	112	140	111	141
18.1	238	234	240	243	240	248	242	320	246	324
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OTALS	932	938	938	973	946	1,024	956	1,488	968	1,521

a C = control; E = experimental cheese